10/530956

J013 Rec'd PCT/PTO 08 APR 2005

APPLICATION

FOR

UNITED STATES LETTERS PATENT

TITLE: THIN-LAYER CELL

APPLICANT: RALF MASUCH

THIN-LAYER CELL

DESCRIPTION

The invention relates to a process for producing a flow

5 cell according to Claim 1.

Flow cells for the measurement of transmission are known
and established in infrared spectroscopy. In such cells,
the liquid sample to be analyzed is passed via sample

10 feeding and removal channels through an optical measuring
chamber which is delimited by infrared-transparent windows.
The IR transmission spectrum measured may be evaluated, for
example, by an FT-IR spectrometer (Fourier transformation
infrared spectrometer). Depending on the application,

15 different designs of such flow cells are used, which may be
configured in a dismantleable and nondismantleable form.

The thicknesses of such cells, i.e. the distance between
the cell windows which delimit the measuring chamber, in IR

20 beam direction are generally between 0.015 mm and several
millimeters. The dimensioning of the cell layer thickness
depends substantially on the IR absorption behavior of the
solvent used. Preference is given to using weakly absorbing
solvents, for example carbon tetrachloride or carbon

25 disulfide.

Water, in contrast, is a problematic solvent in infrared
spectroscopy owing to its strong and wide-ranging
absorption in the infrared spectral region. This is

30 especially true in the middle infrared region of from 4000

1

to 400 cm^{-1}, since water has, for example at 1643 cm^{-1}, pronounced absorption behavior. Even at layer thicknesses of a few micrometers, this results in total absorption of the IR beam in the solvent in certain wavelength regions,

5   so that these regions are analytically inaccessible. For many chemical, biochemical, pharmacological and physiologically interesting substances, water is, however, the natural solvent. The analysis of water-soluble substances is to an ever greater degree at the center of

10 modern scientific and industrial studies and forms the focal point of many biological, medicinal and pharmaceutical investigations.

Owing to the IR absorption behavior of water, the cell

15 layer thickness of flow cells for aqueous samples thus has to be kept small in order to obtain an evaluable transmission signal. The preferred layer thickness for transmission measurements of aqueous samples is, for example, stated to be from 6 to 7 μm by K. Rahmelow and

20 W. Huber in Appl. Spectrosc. 51, 160-170 (1997). In "A stopped flow apparatus for infrared spectroscopy of aqueous solutions", Wharton et al. state that stopped-flow measurements which are usually used to investigate the kinetics of reactions cannot be carried out in transmission

25 cells with the necessary layer thicknesses of approx. 5 μm (erroneously reported as 5 mm in the article). The cause of this is the enormously high flow resistance of such thin-layer measurement cells which is approximately inversely proportional to the layer thickness cubed, as a result of

30 which high pressures arise at layer thicknesses of less

2

than 10 μm.

Known dismantleable flow cells become leaky at relatively high pressures and the risk of cross-contamination of the samples becomes considerably higher. In addition, a uniform layer thickness after a pressurization or after dismantling is no longer ensured in the case of these cells. Natural limits are likewise placed on the layer thickness precision of dismantleable measuring cells by the given environmental conditions, for example dust. Dust particles in air are present mainly in a lower micrometer range, so that a high layer thickness precision can be achieved in the first assembly and in repeated assembly only under dust-free conditions. Reproducible measurements or quantitative determinations are therefore associated with high imprecision.

Known dismantleable flow cells are usually made of silver amalgam or lead amalgam and are designed only for a very low pressure of not more than 2 to 3 bar.

Owing to the above problems, alternative measurement systems for the IR characterization of aqueous samples have been proposed, for example in the form of ATR techniques (attenuated total reflection), in which the infrared beam, owing to physical laws, penetrates into the sample only by a few micrometers, but several times. However, a disadvantage of this technique is that the absorptions are wavelength-dependent and interface effects between optical material and the sample liquid, for example in the form of

adsorption or denaturation of the sample (example: proteins), can occur.

In "Spectroscopy of Biological Molecules: New Directions;
5   8th European Conference on the Spectroscopy of Biological
Molecules", August 29 - September 2, 1999, Enschede, The
Netherlands, edited by J. Greve, G.J. Puppels and C. Otto,
pages 689 to 690, a flow cell was described whose
production comprised a combination of photolithographic
10 structuring techniques for the definition of a spacer
between two IR-transparent windows with subsequent adhesion
of the windows with an epoxy adhesive. Photolithographic
structuring techniques are used in microsystems technology
preferably for the lateral structuring of photosensitive
15 materials (coatings). They are less suitable for precise,
uniform and reproducible material deposition in the form of
a nonlaterally applied spacer layer. This is particularly
true of layer thicknesses in the μm range. Here, this
results in large layer thickness tolerances. Flow cells
20 produced in this way have poorly reproducible flow and
measurement chambers with dead zones and unsatisfactory
plane-parallelism of the optical windows and insufficient
layer thickness precision. Nowadays, IR spectrometers based
virtually exclusively on the principle of Fourier
25 transformation are used, which have distinct advantages
over conventional spectrometers with regard to speed and
measurement precision. There are correspondingly high
demands on the optical sample chamber with regard to
precise manufacture and the associated achievable
30 measurement precision. In particular for the comparability

4

and transferability of measurement results of strongly absorbing samples (for example aqueous solutions) of different measurement cells, a high precision and reproducibility of the layer thickness of the measurement

5   cells and the plane-parallelism of the optical windows are of great significance (see Ramelow: "Fourier-Transformations-Infrarot-Spektroskopie - Von der Signalverarbeitung bis zur Sekundärstrukturbestimmung von Proteinen" [Fourier transformation infrared spectroscopy -

10   from signal processing to secondary structure determination of proteins], HochschulVerlag, 1995). The transferability of measurement results to measurement systems of the same type is gaining ever greater importance with regard, for example, to utilization of spectral databases and the use

15   of automated systems.

In view of the above disadvantages, it is an object of the invention to propose a process for producing high-precision and pressure-resistant flow cells which are suitable in

20   particular for the precise and reproducible determination of strongly absorbing samples, for example of aqueous samples, by means of transmission IR spectroscopy.

The object is achieved by a process having the features

25   specified in claim 1. Preferred embodiments are the subject matter of the dependent claims.

According to the invention, a process for producing a flow cell for the spectroscopic analysis of samples to be passed

30   through comprises the following steps:

5

(a) provision of a first and of a second window, the second window having at least two sample flow channels for supplying and removing the sample to be analyzed;

(b) application of a structured thin layer to one of the

5   windows;

(c) contacting and liquid-tight securing of the thin layer to the other window, in such a way that facing, plane-parallel window surfaces of the windows and the thin layer delimit a flow chamber which is accessible only

10   through the sample flow channels, the windows being optically transparent at least in some regions at least in the region of the flow chamber; and

(d) filling at least some regions of a filling chamber between the windows which is separated from the flow

15   chamber by the thin layer and adjoins the structured thin layer with adhesive.

The inventive flow cell is a nondismantleable thin-layer cell which is produced preferably by using micromechanical

20 or planar-lithographic structuring techniques. The flow cell comprises two, preferably plate-shaped, windows with preferably planar window surfaces, each of which has at least one section transparent in the infrared spectral region. The liquid sample to be analyzed is passed through

25 at least one sample flow channel of the second window (known as the flow head window) into a slot-like flow chamber between facing, plane-parallel window surfaces of the windows. A second sample flow channel allows the sample to be analyzed to be removed from the flow chamber. The

30 flow chamber is optically accessible at least in some

6

regions, so that the sample enclosed between the plane-parallel window surfaces of the two windows can be analyzed spectroscopically in transmission. In this context, for example, a Fourier spectrometer may be used.

5

The process according to the invention for producing the flow cell is notable in particular for the exact and reproducible geometric definition of the flow or sample chamber. To this end, a structured thin layer is applied on one of the windows and delimits the flow chamber along the slot between the plane-parallel window surfaces. The thin layer is preferably applied to one of the window surfaces with micromechanical or planar-lithographic structuring techniques. Subsequently, the other window is brought proximate to the first window, in such a way that the facing planar window surfaces run parallel to one another and the window surface of one window comes into contact with the structured thin layer. The bond between thin layer and window surface is accomplished by the thin layer being secured in a liquid-tight manner to the window surfaces of both windows. The structured thin layer thus forms a liquid-tight barrier layer which is bonded intimately to the window surfaces and delimits the flow chamber in the plane of the slot between the windows. The flow or sample chamber is thus accessible only through the sample flow channels.

The structured thin layer separates the flow chamber from a slot-like filling chamber which is likewise delimited by the plane-parallel window surfaces. For the permanent and

7

exact fixing of the positional alignment of the two windows relative to one another, the filling chamber is filled at least in some regions with adhesive. The adhesive wets the facing window surfaces of the filling chamber and

5 preferably also the structured thin layer delimiting the slot region. After the adhesive has set, a pressure-resistant and reproducible flow cell is provided and is suitable in an outstanding manner for the infrared spectroscopy analysis of aqueous samples. The liquid-tight

10 securing of the thin layer to both window surfaces results in no gap existing between the window surfaces and the thin layer, through which adhesive might penetrate into the flow chamber or into the gap in the subsequent filling step.

15 The function of the sealed flow chamber serves in particular for defined adhesive flow in step (d), so that penetration of the adhesive as a result of capillary forces or under pressure into the flow chamber is prevented. When there is no leakproof bond between the structured thin

20 layer and the second window, penetration of the adhesive into the (nano)gap between structured thin layer and flow head as a result of capillary force cannot be ruled out. This results in an insufficiently defined adhesive interface layer. If the thin layer is to be removed after

25 the adhesive has set, the result in this case would be unsatisfactory flow geometry. In contrast, the process according to the invention enables the formation of smooth interface surfaces of the flow chamber without dead zones.

30 The predefined structure of the adhesive surface (indirect

8

structuring of the adhesive) results advantageously in no demands being made on the adhesive with regard to microstructuring capability. This allows a multitude of adhesives to be used which are optimized according to the

5 demands such as solvent resistance, elasticity or adhesion in particular applications, for example the use of aggressive solvents, high pressure and thermal resistance. Typical adhesives for this application are single-component or multicomponent systems, for example polyamides,

10 polyester urethanes, epoxides, polyacrylates (PMMA), cyanoacrylates, silicones and polyimides. The curing system of the adhesive can be induced to react by light (e.g. UV and/or VIS), heat, atmospheric moisture, or anaerobically by means of metal contact and/or with activator. The

15 activator (applied to one or both sides of the window) may initiate the curing by a free-radical, anionic or cationic mechanism. Combinations are also possible, for example UV + anaerobic curing; this allows, for example, regions to be reached which are not accessible with light and cure by

20 virtue of the secondary system. Adhesives may be elasticized, for example, by addition of long-chain alcohols in order to better compensate stresses in the event of pressurizations. Adhesives may be electrically conductive, in order, for example, to serve as an electrode

25 for electrochemical measurements.

For the production of the windows, preference is given to using IR-transparent materials, for example calcium fluoride, barium fluoride, zinc selenide, zinc sulfide,

30 silicon, germanium, potassium bromide, sodium chloride

and/or silver halide. It is also possible to optimize various materials using polymer coatings for corresponding application requirements, for example water insolubility or biocompatibility. Possible polymers are, for example,

5 Parylene, PTFE and PE.

With the aid of the microstructuring of the thin layer, it is possible to realize any flow geometries which are tailored for particular objectives and ensure a very small,

10 dead zone-free, optical flow and measurement chamber. For example, it is possible to realize flow chambers having a volume of less than 10 nl. This results in a small sample requirement and a lower risk of cross-contamination with the preceding or following sample. In addition, the

15 flushing times and volumes between two measurements can be distinctly reduced. With regard to automated high-throughput use, for example for pharmaceutical screening or in the analysis of expensive samples, for example proteins which are difficult to express, flow cells produced by the

20 process according to the invention thus have considerable advantages over conventional flow cells.

It is possible by the process to produce measurement cells as nondismantleable units which ensure a high pressure

25 resistance and a high resistance toward stresses arising from pressure change in the range from 0 (external vacuum) to 400 bar with extremely short pressure relaxation times. The pressure relaxation time refers to the time which the measurement cell requires in the transition from the

30 pressure-stressed to the unstressed state to recover its

original layer thickness. In this context, the demands on precise measurement cells are at a layer thickness constancy between reference and subsequent sample measurement of less than 1 nm (see Ramelow: "Fourier-

5   Transformations-Infrarot-Spektroskopie   -   Von   der Signalverarbeitung bis zur Sekundärstrukturbestimmung von Proteinen", HochschulVerlag, 1995), so that no errors which can be measured by IR spectroscopy can be detected. For kinetic measurements or for automated high-throughput use

10   for instance, the aim is to attain this state as rapidly as possible (rapid pressure relaxation). Flow measurement cells produced by the process according to the invention are within the lower millisecond range. In contrast, conventional dismantleable measurement cells have a

15   distinctly slower pressure relaxation of a few seconds up to minutes, and a deviation from the original layer thickness (layer thickness before the pressurization) which can be measured by IR spectroscopy generally remains.

In addition, the measurement cells produced by the process

20   according to the invention are guaranteed to be leakproof even at relatively high temperatures, for example when measuring temperature ramps. Conventional dismantleable measurement cells are sufficiently leakproof only up to approx. 60°C. Depending on the selection of the adhesive,

25   the inventive measurement cells may be used from -60°C to +230°C, preferably from +4 to +90°C.

The manufacture of the measurement cells described here by microsystems technology proceeds preferably under cleanroom

30   conditions and ensures a virtually dust-free environment in

the manufacture, which can achieve a particularly high layer thickness precision.

In a preferred embodiment of the process according to the
5 invention, the liquid-tight securing of the thin layer to the other window includes a softening of the thin layer to temporarily lower its viscosity by increasing the temperature of the thin layer and/or by increasing the pressure applied on the thin layer to the other window.
10 Preference is given to achieving the intimate, liquid-tight bond of the thin layer with the window surface by incipiently or fully melting the thin layer. The softening of the thin layer lowers its viscosity in such a way that the window surface of the proximate window is wetted fully
15 by the softened thin layer in order to form an intimate, liquid-tight bond after cooling. In the selection of suitable thin layer materials, a lowering in viscosity may also be brought about by an increase in the pressure applied by the thin layer to the other window. The
20 intimate, liquid-tight bond of the thin layer to the two window surfaces enables a precise and reproducible definition of the flow chamber and a liquid-tight partition of this chamber from the filling chamber.

25 The thin layer should preferably consist of a material which, with regard to the configuration of the flow chamber, enables various structures or microstructures and at the same time permits liquid-tight sealing between the first window (spacer window) and the second window (flow
30 head window). It should preferably be possible to remove

12

the material again by means of a suitable solvent. Conceivable examples of materials are photoresists, lacquers and polymers. For the application and structuring of the thin layer, preference is given to using processes 5 such as photolithography or screenprinting.

In a further embodiment of the invention, the thin layer consists of a viscous material having a viscosity of at least 10 000 mPas at a temperature of 20°C and the liquid-10 tight securing of the thin layer to the other window includes the step of pressing the viscous thin layer onto the other window.

In this embodiment of the process according to the 15 invention, the structured thin layer consists of a comparatively highly viscous material which, when contacted with or pressed onto the window surface to be brought proximate, wets it in such a way that a liquid-tight and gapless bond is formed between thin layer and window. Thus, 20 no adhesive can pass from the filling chamber into the flow chamber through any gap in a subsequent filling step. Alternatively, a low-viscosity material may also be used which, after the gapless bonding between thin layer and window, can be brought into a highly viscous and 25 dimensionally stable state by a further treatment, for example heating (e.g. viscosity increase as a result of solvent evaporation from the material) or illumination. In this context, particular preference is given to using light-curing adhesives which are brought from a low-30 viscosity to a high-viscosity state by the action of light.

13

The adhesives may be applied, for example, by a screen-printing process.

In a particularly preferred embodiment of the invention, a
5   structured spacer layer with predetermined layer thickness
is applied to one of the window surfaces of at least one of
the windows, and the spacer layer comes into contact with
the window surface of the other window in step (c) in such
a way that the distance between the window surfaces is
10 determined by the thickness of the spacer layer. In this
inventive embodiment, a separate spacer layer is applied to
one of the window surfaces in addition to the structured
thin layer which is responsible essentially for the
geometric definition of the flow chamber. The main function
15 of the spacer layer is to ensure a reproducible,
predetermined and plane-parallel separation between the
surfaces of the windows.

For the application of the spacer layer, preference is
20 given to using methods such as sputtering, vapor
deposition, ion plating, PVD, CVD, PECVD, wet-chemical
metal deposition and/or laser-supported material
deposition, which ensure a high precision and
reproducibility with regard to the material deposition.
25 Also suitable to a limited extent are screenprinting
techniques and photolithographic processes. Particularly
advantageous methods are those in which the material
thickness applied can be controlled exactly, so that
predetermined spacer layer thicknesses can be achieved
30 reproducibly. The spacer layer is preferably not deformable

14

thermally or under pressure. In this way, it is possible to adjust the slot thickness of the optical flow chamber reproducibly and precisely.

5   It is possible with the abovementioned preferred production techniques to precisely and reproducibly establish layer thicknesses over a preferred range of from 0.5 to 100 μm, more preferably from 1 to 50 μm and most preferably from 3 to 15 μm. The resulting, likewise high parallelism of the

10 optical windows relative to one another (same layer thickness at each point within the flow cell) enables very precise quantitative analysis of the sample, since disruptive optical effects, for example light scattering, reflection or beam inhomogeneity in the course of beam

15 passage are minimized. The flow cells produced by the process according to the invention thus enable precise calibration and high reproducibility and comparability of measurements which have been taken in different flow cells. The transferability of measurements of identical

20 measurement systems in particular is playing an ever greater role in spectroscopic application, for example spectral databases.

A further significant advantage of the inventive flow cells

25 is the guarantee of uniform liquid flow within the slot-like flow chamber, so that the windows should preferably have high parallelism relative to one another. A wedge arrangement of the windows may lead, in the region of the smallest slot thickness of the slot-like flow chamber, to

30 zones of poor flow (known as dead zones). The spacer layer

15

ensures reproducibly that the planar window surfaces can be aligned plane-parallel relative to one another without the complicated window positioning devices being necessary.

5   Preference is given to applying the spacer layer in an edge region of the window. Such an arrangement of the spacer layer ensures in a particularly efficient manner high plane-parallelism of the window surfaces which are spaced apart. At the same time, the spacer layer is positioned

10 comparatively far from the flow chamber which is preferably formed in the central region of the window surfaces, so that a comparatively large filling chamber is formed. This enables adhesion of the windows over a large surface area by filling of the filling chamber.

15

The thin layer preferably has a complete circular shape. In this case, the circular shape of the thin layer is positioned with respect to the sample flow channels in such a way that they are preferably very close to the thin layer

20 in order to minimize disadvantageous dead zones. With the aid of structuring processes from microsystems technology, it is also possible to construct complex flow geometries which are adapted to the particular requirements, for example the integration of electrodes.

25

At least one of the windows preferably has at least one adhesive channel for feeding the adhesive into the filling chamber. Particular preference is given to an embodiment with two adhesive channels in which the second adhesive

30 channel serves as an adhesive outlet orifice. Particular

preference is likewise given to an embodiment with two adhesive channels in which both channels serve as adhesive inlets and the spacer layer is interrupted at suitable points, these points serving as adhesive outlets. The 5 adhesive is either injected into the filling chamber by pressure application or introduced via the capillary effect.

Preference is given to removing the thin layer after step 10 (d). In the case of thin layers which are solvent-soluble, this may be effected, for example, by flushing the flow chamber with a solvent. The separation of the window surfaces after step (d) is preferably in the range from 0.5 to 100 μm, preferably from 1 to 15 μm and most preferably 15 from 3 to 15 μm.

DESCRIPTION OF THE DRAWINGS

The invention is described by way of example hereinbelow with reference to accompanying drawings of a preferred 20 working example. The figures show:

Fig. 1   production stages of a flow cell during a preferred variant of the production process according to the invention in schematic view 25   (left-hand column) and schematic cross section (right-hand column);

Fig. 2   schematic cross-sectional views of different production stages of a flow cell during one 30   variant of the production process according to

17

the invention; and

Fig. 3   schematic perspective view of a measurement cell
           apparatus comprising an inventive flow cell.

5

DETAILED DESCRIPTION OF THE INVENTION

Fig. 1 (a) to (g) each show, in schematic plan views and
cross-sectional views along the line I-I, essential process
stages of a preferred variant of the process according to
10 the invention for producing flow cells. As shown in
fig. 1(a), an IR-transparent window 10 is used in this
preferred process and has two adhesive channels 12. A
circular spacer layer 16 is defined preferably by
micromechanical or planar-lithographic structuring
15 techniques on a planar window surface 14 of the window 10,
said circular spacer layer 16 preferably consisting of a
material which is substantially incompressible and very
substantially not thermally deformable (for example a
metal) (fig. 1(b)). The layer thickness of the spacer layer
20 16 is controlled in such a way that it corresponds to the
desired later slot separation between the two plane-
parallel windows.

Subsequently, as shown in fig. 1(c), a structured thin
25 layer 18 is applied to the window surface 14 of the window
10. The thin layer 18 has a circular shape and serves to
geometrically define the flow chamber. The layer thickness
of the thin layer 18 is not less than that of the spacer
layer 16, so that it can come into contact with a window
30 surface 20 of a second window 22 when it is moved proximate

to the window 10. In the window 22, which is also referred to as the flow head window, two sample flow channels 24 are formed, through which the liquid sample can be admitted to and discharged from a flow chamber 26. The flow chamber 26

5   is delimited laterally (i.e. in the plane of the slot) by the circular thin layer 18 and connected to the environment only through the sample flow channels 24. The circular thin layer 18 separates the flow chamber 26 from a filling chamber 28 which adjoins the thin layer 18 (fig. 1(d)).

10

After the window surface 20 has been contacted to the thin layer 18 and the spacer layer 16, an intimate, liquid-tight adhesive bond to the window surface 20 is generated preferably by a softening or adhesion or reshaping process

15   of the thin layer, so that no gap remains between the thin layer 18 and the window surface 20. Equally, no gap must remain between the thin layer 18 and the window surface 14 of the window 10 either. In this state shown in fig. 1(e), the filling chamber 28 is separated hermetically and

20   gaplessly from the flow chamber 26.

In this state, as shown in fig. 1(f), an adhesive is injected through one of the adhesive channels 12 into the filling chamber 28 until it is filled fully with adhesive.

25   In the course of this filling, the adhesive fully adjoins the circular thin layer 18. In an optional subsequent step, as shown in fig. 1(g), the thin layer 18 can be removed, so that the flow chamber 26 is now delimited by the adhesive layer in the plane of the slot. In fig. 1, the adhesive is

30   emphasized by gray shading.

19

In fig. 2, the sequence of steps shown in fig. 1(a) to (c)
in a particularly preferred embodiment is shown once again
in detail. The spacer layer 16 is initially applied with a
5   defined layer thickness to the window 10, shown in
fig. 2(a), for example by means of screenprinting
technology or a vapor deposition or sputtering technique
and lithographic structuring. Subsequently, the window
surface 14 is coated with a photoresist layer with defined
10   layer thickness by means of a spincoater. In this context,
the resist layer thickness is slightly greater than that of
the spacer layer 16. Fig. 2(d) shows the finished
structured thin layer 18 after completion of the subsequent
lithography and removal step.

15

The finished measurement cell apparatus (the flow head) is
constructed preferably in the form shown in fig. 3. A metal
plate 30 with at least two drillholes for the sample flow
channels 12 and with at least one cutout 32 for the optical
20   beam path is preferably bonded to the window 22 in such a
way that the drillholes of the metal plate 30 are
preferably bonded to one another with precise fitting or
via channels in the window 22 or in the metal plate 30 or a
sealant material 34. The sealing between the window 22 and
25   the metal plate 30 may be effected by adhesive bonding or
by sealants 34. The connections to the inlets and the
outlets may be such that precisely fitting capillaries 36
are conducted through the drill holes of the metal plate
30, of the sealant material 34 and of the window 22, and
30   are ended by HPLC connections which are present in the

20

metal plate 30 or are pressure-tight and substantially dead zone-free by virtue of adhesive bonding. The capillaries 36 preferably end planar to the window underside 20, i.e. end directly before the thin layer.

5

REFERENCE NUMERAL LIST

10   window (spacer window)

12   adhesive channels

5   14   window surface of the window 10

16   spacer layer

18   structured thin layer

20   window surface of the window 22

22   window (flow head window)

10   24   sample flow channels

26   flow or sample chamber

28   filling chamber

30   metal plate

32   cutout

15   34   sealant material

36   capillaries